Phytochemical Investigation of Select Ayurvedic Medicinal Plants and Biological and *in silico* Validation of Anti-Inflammatory Potential

Submitted By

P Rajasri Bharathi

17PHCHF004

Supervisor

Dr. Shubashini K Sripathi

Department of Chemistry

Submitted to

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore – 641 043

March 2022

Phytochemical Investigation of Select Ayurvedic Medicinal Plants and Biological and *in silico* Validation of Anti-Inflammatory Potential

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. Plants are indispensable to man for his life. Nature has provided a complete storehouse of remedies to cure all ailments of mankind. In the past, almost all the medicines used were from plants. Medicinal plants represent the eternal kindness of almighty for the perpetuation of life in the universe. In terms of life forms, medicinal plants are equally distributed across habits, viz, trees, shrubs and herbs. The history of the use of plants as a primary source of medicine can be traced back several millennia to the ancient written documents of early civilizations of India, China and the Middle East. The Charak Samhita written during 990BC has documented the production of around 340 herbal drugs and their indigenous uses for curing various ailments and diseases **(Rahul *et al.*, 2010).**

India is one of the twelve biodiversity centres of the World. Medicinal plants have been selected by the Indian government as one of the thrust areas of the global economy. The Ministry of Environment and Forests, Government of India, has reported that there are over 8000 species of medicinal plants grown in the country. According to a study on the export of Indian medicinal plants, 880 medicinal plant species find place in All India Trade but India's share is only 0.5 % in global medicinal plant related export trade **(Ramesh *et al.*, 2011).** Owing to the fact that the Indian subcontinent is a bountiful source of medicinal plants and has a rich heritage of knowledge on medicinal plant use for curing diseases, validation and standardisation of medicinal plants and their products will fetch huge returns in the global market which is big and expanding. Based on this revelation, during the past decade, a large number of plant species have been taken up for validation studies and systematic documentation of data is being done. Therefore, any herb-based research must have an accompanying validation and standardization platform. Validation and standardization of medicinal plants has been mightily emphasized by the World Health Organization which has laid down recommendations for standardization **(WHO 2007).**

1. Medicinal Plant Chosen for the Study

In Indian traditional Ayurvedic medicine, ***Jathyadi Thailam*** finds use as a wound healing medicine. *Jatyadi* is a therapeutic drug used to treat wounds, diabetic chronic wounds, ulcers, fistulas, fissures, dermatitis, eczema, scalds, and burns **(Mandrika *et al.*, 2021).** *Jathyadi Thailam* as formulated by Arya Vaidya Pharmacy consists of thirteen herbs that are fortified in coconut oil. In the present research work, three constituent plants of

Jathyadi thailam were chosen for phytochemical profiling and one plant namely *Jasminum flexile* was subjected to chemical investigation for isolation of compounds. The plants chosen are

Plant I: ***Jasminum flexile* Vahl**. of plant family ***Oleaceae***

Plant II: ***Physalis minima* Linn**. of plant family ***Solanaceae***

Plant III: ***Calycopteris floribunda*** (Roxb.) Lam. of plant family ***Combretaceae***

The plant ***Jasminum grandiflorum***, a species close to *Jasminum flexile* and belonging to *Oleaceae* family was also phytochemically profiled with data reported in literature.

The research study will be presented in five chapters.

Chapter I comprises of a brief introduction to the study and a mention of its prime focus.

Objectives

- To carry out phytochemical profiling of the chosen Ayurvedic medicinal plant *Jasminum flexile* Vahl
- To isolate and characterize chemical constituents from the leaves of *Jasminum flexile* by radial chromatography and column chromatography
- To validate the biological potential of the chosen plant extracts, fractionates and compounds isolated from the leaves of *Jasminum flexile*
- To carry out *in silico* studies with predicted molecules of GC-MS analysis of *Jasminum flexile*
- To carry out *in silico* studies with lead molecules of *Jasminum grandiflorum* against antibacterial and anti-inflammatory targets
- To prepare a ready reckoner documentation sheet for the plants *Jasminum flexile*, *Jasminum grandiflorum*, *Physalis minima* and *Calycopteris floribunda*

Review of literature pertaining to the study will comprise Chapter II

Exhaustive search of literature was done for the plants

Jasminum flexile Vahl was reviewed for the period 1985 to 2021.

Jasminum grandiflorum Linn was reviewed for the period 1958 to 2021.

Physalis minima was reviewed for the period 1974 to 2021.

Calycopteris floribunda was reviewed for the period 1934 to 2021.

The review revealed that except *Jasminum flexile* the other plants have been well investigated. Hence, *Jasminum flexile* was selected for the phytochemical investigation. A review of iridoids for the period 2000 to 2021 will also be presented in the thesis since, plants of the genus *Jasminum elaborate* a number of iridoids.

Chapter III

The methodology adopted for the research work comprising of five major phases will be presented in Chapter III.

Phase I: Phytochemical profiling of leaves of *Jasminum flexile* Vahl

Phase II: Chemical investigation of leaves of *Jasminum flexile*

Phase III: Biological activity studies

Phase IV: *in silico* studies

Phase V: Preparation of documentation sheet for chosen Ayurvedic plants

Bulk Extraction of Plant Material

The respective plant material was authenticated and extracted successively with hexane and hydroethanol (90:10 Ethanol-water mixture) solvents. Two successive extractions of six hours duration each was carried out. Each extract was concentrated in vacuum. The extract concentrates are designated as given below

JAFL (H) Hexane extract of *Jasminum flexile*

JAFL (HE) Hydroethanolic extract of *Jasminum flexile*

PHMI (H) Hexane extract of *Physalis minima*

PHMI (HE) Hydroethanolic extract of *Physalis minima*

CAFL (H) Hexane extract of *Calycopteris floribunda*

CAFL (HE) Hydroethanolic extract of *Calycopteris floribunda*

The above extracts were taken up for the phytochemical profiling and chemical investigation studies as the case may be.

Phase I: Phytochemical Profiling of Leaves of *Jasminum flexile* Vahl

The stages of work involved in the phytochemical profiling will be presented in the following sections

- **Physico-chemical characterization**
- **Proximate analysis**
- **Phytochemical characterization**
- **Spectral fingerprinting of extracts**
- **Chromatographic fingerprinting of extracts**

Standard methods were adopted for the documentation of data pertaining to the above.

Phase II: Chemical Investigation of Leaves of *Jasminum flexile*

Isolation and Characterization of Chemical Constituents from the *Jasminum flexile*

Isolation chemical constituents from the leaves of *Jasminum flexile* was done as outlined in Charts 1 and 2. The hexane extract of leaves of *Jasminum flexile* was subjected to cyclograph-aided radial separation. The hydroethanolic extract of leaves of *Jasminum flexile* was analysed by column chromatography for separation of constituents.

Cyclograph-Aided Radial Separation

The hexane extract of ***Jasminum flexile*** [JAFL (H)] was dissolved in the minimum volume of hexane and sonicated. The sonicated extract to be separated was applied on the adsorbent rotor by passing it through with a solvent pump. Initially, 100% hexane followed by solvent mixture of hexane and ethyl acetate in the ratio of 7:3 was pumped through the adsorbent layer effectively. As the eluant reached the outer rim of the rotor, it was collected. The collected fractions were examined by TLC. The spots were visualized under UV light and then by exposure to iodine vapours. Similar fractions were combined and purified.

Column Chromatographic Isolation of Chemical Constituents

The extract concentrate of *Jasminum flexile* was dissolved in minimum volume of methanol and made into slurry with minimum amount of silica gel. The slurry was subjected to chromatographic separation over a column of silica gel (100 g) built in hexane (H) and eluted with increasing amounts of ethyl acetate (EA). Eluates of 100 ml were collected each time and the solvent was distilled off on a water bath. The homogeneity of the fractions was examined by TLC. The spots developed were visualized under UV light and then by exposure to iodine vapour. Similar fractions were combined and purified.

Further as part of the chemical characterization studies, the column main fractions and cyclograph main fractions have also been subjected to GC-MS and LC-MS analysis and the probable compounds identified from the fractionates.

Characterization of Isolated Compounds

The isolated compounds were characterized by preliminary tests, physical data and spectral analysis. Spectral techniques used for structure elucidation include UV, IR, 1D-NMR, 2D-NMR and Mass spectrometry.

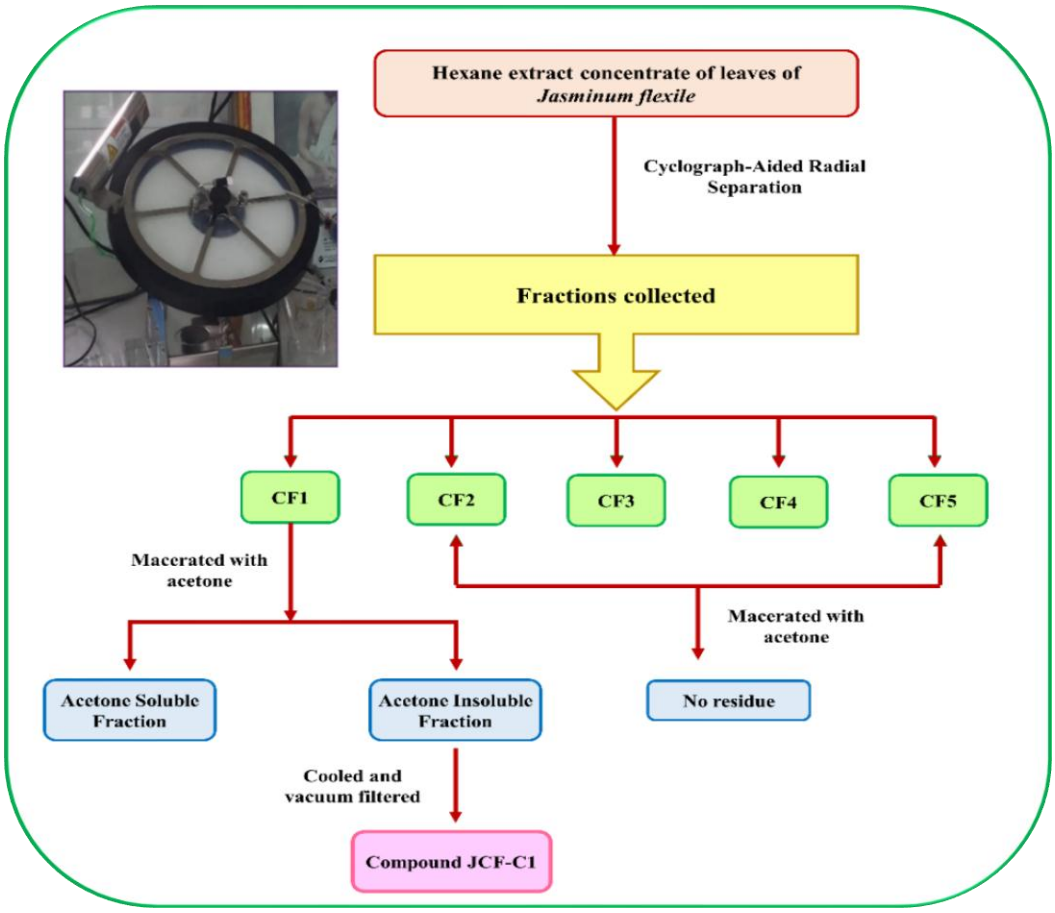

Chart 1: Outline of Procedure for Isolating Components from the JAFL (H)

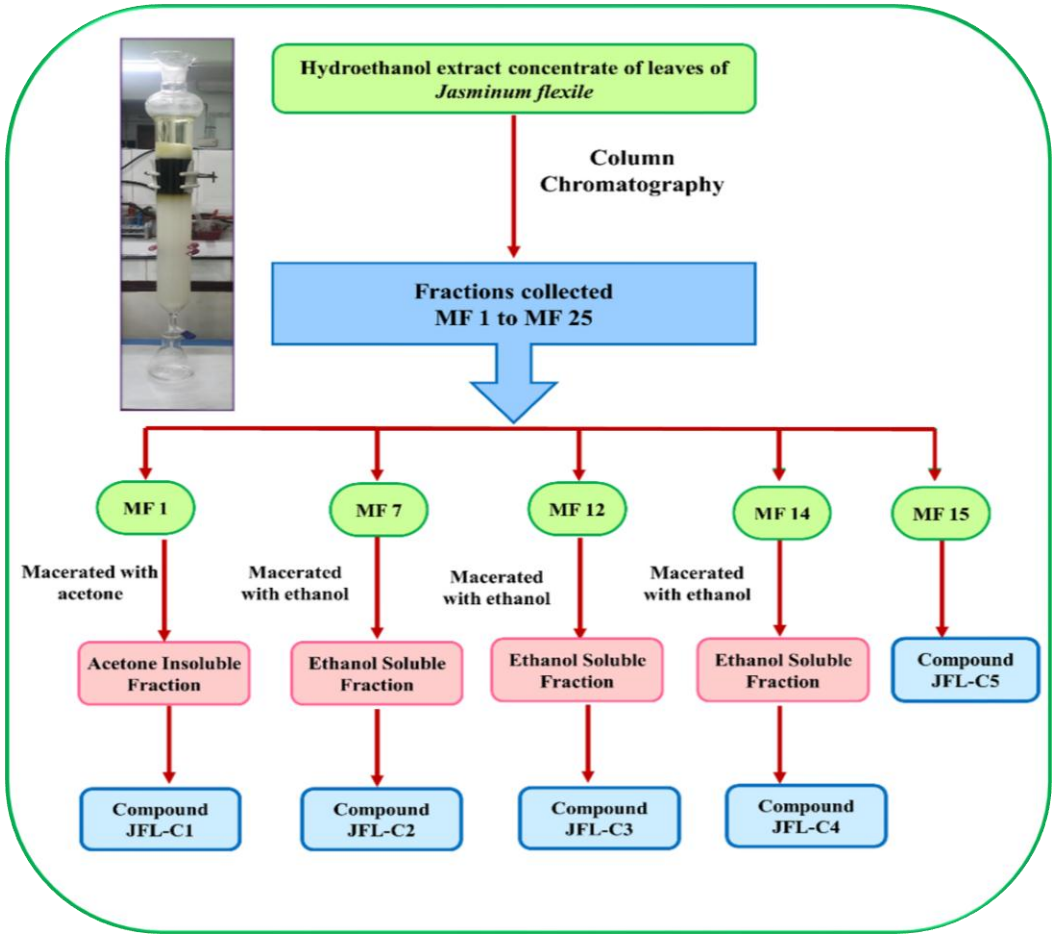

Chart 2: Outline of Procedure for Isolating Components from the JAFL (HE)

Phase III: Biological Activity Studies

The sample (10 mg) was dissolved in 1ml of DMSO and incubated at 40 ºC in a shaker incubator for 24 hours. After incubation, the sample was taken up for ***in vitro*** **studies.** Antibacterial and Anti-inflammatory assays **(NCCLS, 1993; Elias and Rao, 1998; Seeger, 1980)** was done for

- Leaf extracts of *Jasminum flexile*
- Cyclograph main fractions of *Jasminum flexile* hexane extract
- Column main fractions of *Jasminum flexile* hydroethanol extract
- Isolated compounds

Phase IV: *in silico* Studies

The structure of compounds was docked with the 3D structures of differentially expressed protein products of the genes and the interaction strengths were accomplished using Schrodinger 2018 Drug Designing Software. *In silico* studies were done for

- **Compounds predicted by GC-MS analysis of *Jasminum flexile* extracts**
- **Select chemical compounds reported earlier from *Jasminum grandiflorum***

From the results of the preliminary *in silico* studies of the compounds of *Jasminum grandiflorum*, thirty compounds were selected for "optimization studies" using Gaussian 16 software using the Hartree-Fock method with hybrid exchange-correlation functional 3-21G. For the *in silico* studies the antibacterial targets chosen are 1DDR and 7RRJ. For the anti-inflammatory targets chosen are 5WBE, 3NTG, 4YL3, 5IKQ, 5UUI, 4O9H.

Phase V: Preparation of Documentation Sheet for Chosen Ayurvedic Plants

All reported data on four plants *(Jasminum flexile, Jasminum grandiflorum, Physalis minima, Calycopteris floribunda)* as well as the findings of this study, are documented as a ready reckoner sheet, which will aid herbal manufacturers in developing standardized formulations. This will increase the validity of the herbal product labels, thereby increasing the global market value of formulations **(Nikam *et al*., 2012; Ekka *et al*., 2008; Rajani and Kanaki, 2008)**. The documentation template for each plant will comprise of

- The complete phytochemical profiling details of each plant
- The marker/biomarker quantification protocols
- Toxicity data

The results pertaining to the present work titled "**Phytochemical Investigation of Select Ayurvedic Medicinal Plants and Biological and *in silico* Validation of**

Anti-Inflammatory Potential" and the ensuing discussions will be presented in **Chapter IV.** Highlights of the results and their significance will be presented below.

Chapter IV

Phase I: Phytochemical Profiling of leaves of *Jasminum flexile* Vahl

Physicochemical Characterization

Organoleptic Properties Organoleptic analysis revealed that the chosen *Jasminum flexile* are suitable for use as an Ayurvedic medicine. The results will be present in the thesis.

Solubility - The JAFL (H) extract was insoluble in water while the JAFL (HE) extract was soluble in most of the solvents.

Fluorescence Analysis - The dried powdered parts of leaves of *Jasminum flexile* depicted fluorescence with chemical reagents under day light and UV light indicating that the selected plant parts are source of natural metabolites.

Elemental Analysis - The content of carbon, oxygen and hydrogen were found to be highest in the leaves of *Jasminum flexile*. EDX analysis for both the samples revealed the absence of toxic elements, thus proving that both the samples are most suitable for biological applications.

Proximate Analysis - The results indicated that the leaf part are a good source of carbon, minerals and fibre.

Extractive Value - A higher extractive value of hydroethanolic extract of leaves of *Jasminum flexile* indicated a higher percentage of polar metabolites present in the leaves.

Phytochemical Characterization

Preliminary Qualitative Color Tests

The hexane extract indicated the presence of steroids and terpenoids and the hydroethanolic extract tested positive to alkaloids, saponins and phenolic compounds. Both extracts were positive to terpenoids and steroids.

Quantification of Phytoconstituents

The content of phytoconstituents in *Jasminum flexile* leaves is tabulated in table 1 below. The results indicate that this plant material is rich in steroids and terpenoids.

Table 1: Quantification of Phytoconstituents from the leaves of *Jasminum flexile*

Phytochemical	Phytochemical Content (%)
Alkaloids	0.12
Steroids	3.5
Terpenoids	2.7
Phenolic Compounds	0.33
Flavonoids	0.77

Spectral Fingerprinting of Extracts

The UV-Visible, FTIR and NMR spectra of JAFL (H) and JAFL (HE) were recorded to document as fingerprints of the leaf extracts.

Chromatographic Fingerprinting of Extracts

TLC Analysis - An optimized condition for TLC analysis was achieved for the various extracts with solvent mixtures.

Hexane - ethyl acetate mixture (7:3 V/V) for JAFL (H) extract

Hexane - ethyl acetate mixture (8:2 V/V) for JAFL (HE) extracts

GC-MS Analysis - GC-MS analysis of hexane and hydroethanolic extracts was done. The peaks with intensity greater than 10% in the total ion chromatogram were considered. The mass spectrum corresponding to each fragment was compared with that given by NIST database. Peak by peak comparison of the mass spectra gives the probable compounds for each. In JAFL (H), seven compounds and in JAFL (HE) four compounds were predicted.

Phase II: Chemical Investigation of Leaves of *Jasminum flexile*

Cyclograph-Aided Radial Separation

Cyclograph-Aided radial separation of hexane extract concentrate of leaves of *Jasminum flexile* led to the isolation of **one compound**. Further compounds will be identified from the GC-MS results. The characterisation of the compound is under progress and the data will be presented in the thesis.

Column Chromatographic Isolation of Chemical Constituents

Column chromatographic analysis of hydroethanolic extract concentrate of leaves of *Jasminum flexile* led to the isolation of **five compounds**. Totally 172 fractions were collected by column chromatographic analysis which led to 25 main fractionates. The compounds isolated and the main fractionates will be characterized and the results presented in the thesis.

Phase III: Biological Activity Studies

Anti-bacterial Activity Studies

Leaves of *Jasminum flexile* have not been explored extensively for its biological potential. Only one report on anti-oxidant potential of leaves of *Jasminum flexile* has been reported **(Sulaiman *et al.*, 2013).** Hence, the extract concentrate of leaves of *Jasminum flexile,* cyclograph fractionates, column fractionates and isolated compounds were standardized by biological activity studies.

The hexane extract exhibited higher antibacterial activity compared to hydroethanolic extract. Cyclograph fractionates expressed moderate antibacterial activity compared to the

standards Ampicillin, Kanamycin and Chloramphenicol. The compound isolated by cyclograph-aided radial separation JCF-C1 shows moderate antibacterial activity in comparison with the standard. Among the column fractionates, fractions MF 14, MF 9, MF 5, MF 15 exhibited higher zone of inhibition against *S. mutans* and MF 1, MF 3, MF 4, MF 5, MF 11 exhibited significant zone of inhibition against *K. pneumoniae*. Fractions MF 6, MF 7, MF 10, MF 14 and the total extract JAFL (HE) were active against *E. coli*. Among the compounds isolated JFL-C1, JFL-C2 and JFL-C4 exhibited higher zone of inhibition against *E.coli* and *S. mutans.*

Anti-inflammatory Activity Studies

Anti-inflammatory activity of extracts, fractionates and isolated compounds were carried out by a haemolysis assay. Cyclograph hexane fractions shows moderately significant anti-inflammatory activity compared to standard diclofenac. Column main fractions shows maximum percentage of anti-inflammatory potential for MF 3 (55.14%) followed by MF2 (54.73%), MF 24 (54.04%) and MF 14 (54.08%), whereas the parent hydroethanol extract JAFL (HE) extract showed 51.01%. The compounds JFL-C1 JFL-C2, JFL-C3 show higher percentage of anti-inflammatory inhibition than other compounds.

The overall results revealed that the plant *Jasminum flexile* has good anti-inflammatory properties. Since it is one of the constituents of *Jathyadi thailam* which is said to have wound healing potential, this study validates the contribution of *Jasminum flexile* leaves to the anti-inflammatory potential of the thailam.

Phase IV: *in silico* Studies

The *in silico* studies provided the following revelations.

Among the compounds predicted by GC-MS analysis of *Jasminum flexile* extracts, spiro[androst-5-ene-17,1'-cyclobutan]-2'-one,-hydroxy-,(3.beta.,17.beta.)- gave high docking score with both antibacterial and anti-inflammatory targets.

Among the compounds of *Jasminum grandiflorum*, the iridoids namely 10-hydroxy-ligstroside and 8-dehydroxy shanzhiside, the flavonoid butin-7-O-β-D-glucopyranoside and small molecules oleacein and 2-(3,4-dihydroxyphenyl)ethanol exhibit high anti-inflammatory properties as evidenced from their low binding energy and high docking score. It was observed that among the flavonoidal compounds, compounds which possessed a 3',4'-dihydroxy phenyl moiety as the C-ring of the flavone, the presence of secoiridoidal moiety with hydroxyl group at the C-10 position and the 3,4 dihydroxy phenylethyloxy moiety in iridodial compounds and the 3, 4, dihydroxy phenylethyloxy moiety in small molecules imparted good molecular interaction as evidenced from the docking score.

Phase V: Preparation of Documentation Sheet for Chosen Ayurvedic Plants

A ready reckoner document sheet for four Ayurvedic medicinal plants similar to the Ayurveda plant monograph is one of the outcomes of the study and will be presented in the thesis.

Conclusion

The research study is done to validate the anti-inflammatory potential of the leaves of aroma plant *Jasminum flexile* Vahl. This is the first study on isolation of constituents from leaves of this plants.

Cyclograph-aided radial separation of hexane extract concentrate of leaves of *Jasminum flexile* led to the isolation of one compound. Column chromatographic analysis of *Jasminum flexile* hydroethanol extract led to the isolation of five compounds. Anti-bacterial and *in vitro* anti-inflammatory assays were done for total extracts (hexane and hydroethanol) of leaves of *Jasminum flexile,* the main fractions obtained by cyclograph-aided radial separation of hexane extracts of leaves, the main fractions obtained by column chromatographic separation of hydroethanolic extract of leaves and compounds isolated. *in silico* studies on the compounds predicted by GC-MS analysis of hexane and hydroethanolic (90:10) extracts of leaves of *Jasminum flexile* was attempted. Further to the substantiate *in silico* studies, the phytoconstituents earlier isolated compounds from a similar species namely *Jasminum grandiflorum* was done exhaustively using advanced computational Gaussian 16 software and Schrodinger maestro *v*.11.8 software. The results of the study lead to the identification of pharmacophore moiety of the chemical markers of *Jasminum grandiflorum.* A ready reckoner document sheet for four Ayurvedic medicinal plants was prepared which will comprise of all phytochemical profiling, quantification of biomarkers and toxicity studies. This document will aid herbal formulation manufacturers in standardizing the ingredient herbal extracts.

The research study led to isolation of constituents of leaves of *Jasminum flexile* Vahl and contributed to the validation of its herbal potential. The complete results and discussion will be presented in the thesis.

Paper Publications

1. *Jasminum grandiflorum* Linn. – An Update Review, Rajasri Bharathi P and Shubashini K Sripathi: International Journal of Pharmaceutical Sciences and Research 2020; 11(5): 1994-2010.
2. A Ready Reckoner Documentation Sheet on Standardization Studies on *Calycopteris floribunda* (Roxb.) Lam. – An Overview, Rajasri Bharathi P and Shubashini K Sripathi: International Journal of Biology, Pharmacy and Allied Sciences 2021; 10(11): 4225-4247.

Paper Accepted

1. Physicochemical Analysis, Fingerprinting and Antioxidant Potential of Leaf Extracts of *Jasminum flexile* Vahl., Rajasri Bharathi P and Shubashini K Sripathi: Bulletin of Environment, Pharmacology and Life Sciences 2021; 10: 1-25.

Paper Communicated

1. Computational Investigations to Identify Selective Inhibitors Towards the Development of Natural Product Based Anti-inflammatory Therapy.

Acknowledgment

The authors wish to thank the Avinashilingam Institute for Home Science and Higher Education for Women for providing facilities for current research work. We extend our thanks to the Arya Vaidhya Pharmacy (AVP Pvt Ltd.), Coimbatore, India for providing plant materials.

References

1. Ekka NR, Namdeo KP, Samal PK. Standardization Strategies for Herbal Drugs-An Overview. Res J Pharm Tech 2008;1(4):310-312.
2. Elias G, Rao MN. Inhibition of Albumin Denaturation and Anti-Inflammatory Activity of Dehydrozingerone and Its Analogs. Indian J Exp Biol 1998;26(10):540-542.
3. NCCLS (National Committee for Clinical Laboratory). Performance Standards of Antimicrobial Disc Susceptibility Test, Sixth ed., M2 – A6, Wayne, PA,USA.
4. Nikam PH, Kareparamban J, Jadhav A, Kadam V. Future Trends in Standardization of Herbal Drugs. J Appl Pharma Sci, 2012;2(6):38-44.
5. Rahul C, Lincy J, Methew G and Pankaj P. Pharmacognostic Standardization and Phytochemical Screening of *Albizzia lebbeck*. J Chem Pharm Res 2010 2(1): 432-443
6. Rajani M, Kanaki NS. Phytochemical Standardization of Herbal Drugs and Polyherbal Formulations. Bioactive Mol Med Plants 2008;349-369.

7. Seeger R. Studies on Rubescenslysin Haemolysis. Naunyn-Schmiedeberg's Archives Pharmacol 1980;311(1):95–103.
8. Sulaiman CT, Soudha V, Deepak M, Balachandran I. Comparative Phytochemical Studies and Evaluation of Radical Scavenging Activity in Selected *Jasminum* Species. Int J Pharmacognosy Phytochem Res 2013;4:99-104.
9. WHO Guidelines on Good Manufacturing Practices for Herbal Medicines, 2007.